Mycol. Res. **96** (2): 125–127 (1992) *Printed in Great Britain*

Persistent meiotic arrest in basidia of *Agaricus bisporus*

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The pattern of distribution of genetic markers among progeny of *Agaricus bisporus*, whether conventional mutants, electrophoretic isozyme variants or restriction fragment length polymorphisms, implies a combination of reduced recombination rates and preferential segregation of centromeres during meiosis. We have considered an alternative explanation, namely that meiosis may be an infrequent event, the majority of basidiospores being produced by mitosis. By examining the dynamic population structure of nuclear division in the *A. bisporus* hymenium over the whole of the development of the mushroom fruit body, we find that meiosis is common. Fusion nuclei were readily observable and were in a large majority. However, basidioles with single fusion nuclei remained the majority class throughout the life of the fruit body, right through to senescence. This suggests that release from meiotic arrest is a rare event and some of the observed peculiarities in segregation patterns might be a consequence.

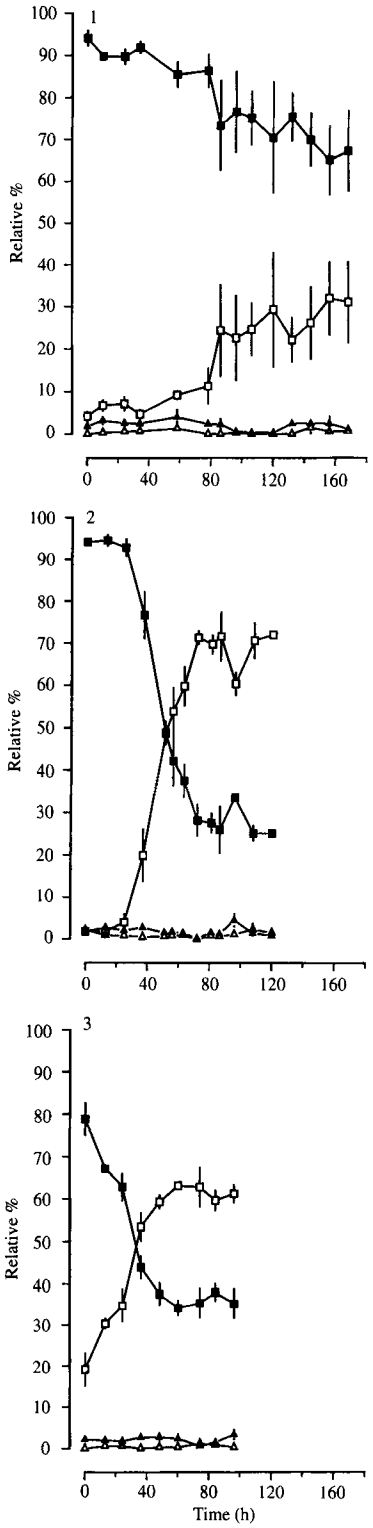
The cultivated mushroom, *Agaricus bisporus* (Lange) Imbach poses many difficulties for geneticists. The mycelium is slow growing, spore germination is poor and few marker genes exist for use in crosses. The majority of improvements to the crop have been made as a result of chance variants and improved cultivation rather than through a systematic breeding programme. Most commercial strains have been derived from multispore cultures where a mass of spores from a spore print are germinated together. A very few hybrid cultures have been bred which combine the best features of parental homokaryons and these hybrids currently dominate mushroom sales in Europe and America, illustrating the benefits to be gained from a systematic breeding method.

Fundamental to such a method would be a full understanding of the process of meiosis as this determines gene segregation patterns in hybrids. Though it might be assumed that normal meioses occur in the basidia of *A. bisporus*, resulting in the production of four nuclei which then migrate to the two spores, recent work in this laboratory and elsewhere on the segregation of restriction fragment length polymorphisms (RFLPs) leads to the conclusion that both chromosome segregation and gene recombination are extremely rare.

Raper, Raper & Miller (1972) and Raper & Raper (1972) used auxotrophic mutants to demonstrate segregation and recombination in the progeny of crosses between their *A. bisporus* strains. Clear phenotypic ratios were not obtained but the authors felt that any alternative to a true sexual cycle to explain their results would, 'invoke improbable combinations of rare phenomena (e.g. vegetative diploidy, aneuploidy, mitotic recombination, haploidization etc.) supported by no direct evidence.' May & Royse (1981, 1982) and Royse & May (1982) searched for isozyme variation amongst lines of *A. brunnescens* (= *bisporus*). Only 5 genotypic classes were observed among 34 commercial lines, the paucity of genetic variation suggesting that the commercial mushroom may be a near monoculture. Few recombinants were discovered. The authors suggest that their findings 'support the contention that low levels of meiotic recombination occur and the chromosomal segregation is non-random in *A. brunnescens*'.

Castle, Horgen & Anderson (1987) studied RFLPs in *A. brunnescens* and *A. bitorquis*. Most cloned fragments were polymorphic within each species but two probes were not polymorphic within *A. brunnescens* at all. Four of the commercial strains exhibited identical patterns of hybridization with all ten probes. The work of Loftus *et al.* (1988) also revealed very little genetic variation within *A. bisporus*. Differences in RFLP pattern were mostly between traditional and hybrid cultivars. If the traditional strains are considered alone, variation was found for only two out of ten probes prepared from anonymous fragments (i.e. sequences chosen at random from a genomic library). In our own experiments (Allen, unpublished), when nine anonymous probes were used to analyse up to thirty progeny, no segregation of polymorphism was found. This result concurs with that of Summerbell *et al.* (1989) who studied RFLP segregation in 367 single-spore progeny from seven parental strains. A total of 351 (95·6%) of the progeny were heteroallelic at all RFLP loci heteroallelic in the parents. The authors conclude that 'meiosis in *A. brunnescens* is accompanied by low levels of recombination' and that 'non-sister nuclei are preferentially incorporated into basidiospores after meiosis II'.

Overall, therefore, genetic analysis using both conventional and molecular markers reveals a surprising lack of segregation and recombination during the formation of basidiospores in



Figs 1–3. Numbers of nuclei observed in hymenial cell populations of *Agaricus bisporus*. Each figure shows data (mean ± S.D.) from three fruit bodies chosen so that at the start of the observations each primordium cap was about 1 cm in diam. Open squares show basidioles containing one nucleus, closed squares those containing two, closed triangles = three nuclei and open triangles = 4 nuclei. Figs 1 and 2 show first flush fruit bodies; Fig. 3 shows fruit bodies from the second flush.

A. bisporus. It can be argued that the phrases 'low levels of recombination' and 'preferential centromere segregation' describe mitosis, and that the segregation data and cytological observations could be reconciled by a situation in which most basidiospores arose through mitotic division. This is not to say that meiosis would be absent: meiotic figures have been visualized by conventional light microscopy (Evans, 1959) and there is a record of synaptonemal complexes being observed by electron microscopy although these are restricted to 'several very short pieces' (Fletcher, 1981). To reconcile the cytology and genetics it could be envisaged that the two-spored phenotype is conferred by a 'mutant' which causes the majority of basidiospores to be produced by mitosis. This would explain the overwhelming number of parental-type heterokaryons found by ourselves and others. If penetrance of the 'variant' which causes this is incomplete, rare meioses could occur to generate those few segregants and recombinants which are observed.

We know of no research aimed at establishing the frequency or rate of progress of meiotic divisions in hymenia of any basidiomycete. In this study we have examined the dynamic population structure of nuclear division in the *A. bisporus* hymenium over the whole of the development of the mushroom fruit body. We find that karyogamy occurs in the majority of basidia, thus ruling out the possibility that conventional mitosis is a major contributor to the formation of daughter spores. However, progress through meiosis is slow and even in old fruit bodies, at any one time only a very small minority of basidia have four daughter nuclei, suggesting that in most basidioles meiosis is arrested in meiotic prophase.

METHODS AND MATERIALS

Fruit bodies were obtained from two commercially available mushroom growing kits, treated according to the supplier's instructions. Three different studies were made; two studies were made on first-flush fruit bodies and one on second-flush fruit bodies. Each study used three fruit bodies chosen so that at time zero each primordium cap was about 1 cm in diameter.

Samples were thin segments of gill tissue, which included some of the deep immature gill layer, that were removed from fruit bodies with a scalpel and fixed immediately in 4% formaldehyde (prepared from paraformaldehyde and adjusted to pH 8·2). Successive samples were taken at approximately 10 h intervals from each fruit body as it continued to develop. Each sample was silver-stained by the method described by Pukkila & Lu (1985) but it was found that, for *A. bisporus*, staining times of between 5 and 15 min were required for nuclei to be stained clearly (depending on the age of the fruit body when the sample was taken); this compares with a staining time of only 2 min for basidia of *Coprinus cinereus*. Stained sections were observed by bright field microscopy. At least 100 basidial cells were scored for the number of nuclei they contained in each of three samples at each sampling time. Observations continued for up to 200 h.

As the fruit bodies aged, it became more difficult to squash and to stain them satisfactorily. At the start of the observation period it was possible to score all the cells visible for numbers of nuclei, but in samples taken from fruit bodies about one week old perhaps only 1 in 10 cells in the field of view could be scored accurately. For this reason, only data from samples taken from fruit bodies up to 170 h old are illustrated.

RESULTS AND DISCUSSION

Results are displayed in Figs 1–3. These show that although the exact timing of events varied, there was a consistent trend for the proportion of cells with a single (fusion) nucleus to increase rapidly during fruiting (there being a corresponding decline in the proportion of dikaryotic basidioles). In all nine specimens the proportion of uninucleate cells remained high during the whole of the life of the fruit body.

As in most other agarics, the *A. bisporus* hymenium lacks differentiated structural cells. The hymenium seems, rather, to be made up of cells indistinguishable from young basidia (= basidioles) which are continually produced to replace those which mature and sporulate, thereby enabling the fruit body to produce spores gradually over the 5–7 days that it is viable (Buller, 1922; pp. 405–435). In such a 'classic' agaric hymenium, successive generations of basidia are seen as serving a structural function during their young stages.

Our observations suggest that this is achieved through meiotic arrest causing a prolonged meiotic prophase. They also suggest that far more basidioles enter meiosis than can ever complete it, so that, for a very large number of basidioles, the state of arrest in meiotic prophase represents the terminal stage of differentiation. Basidioles with a single fusion nucleus became the majority class rapidly and remained so right through to senescence of the fruit body; this suggests that release from meiotic arrest occurs only rarely. If progress through meiosis is indeed subject to tight control then some of the observed peculiarities in meiotic segregation patterns might be a consequence of residual effects of this.

The original objective of these observations was to decide whether it is meiosis or mitosis which is responsible for the production of daughter nuclei for the two spores of *A. bisporus*. Fusion nuclei were very readily observable and were in a large majority. We therefore conclude that meiosis is not an infrequent event and that the lack of detectable genetic segregation in basidiospore progeny does reflect controlled reduction in recombination frequencies coupled with selective segregation of centromeres. Whether these features are themselves correlated or associated with the meiotic arrest, secondary homothallism and formation of only two basidiospores has yet to be established.

We thank SERC for a CASE studentship, and Diatech Limited for their financial support of this project.

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(*Accepted 5 September 1991*)